

Foliar Nutrient Concentrations and Antioxidant Activity of Tea (*Camellia sinensis* L. (O) Kuntze) Planted in Peninsular Malaysia and its Relation to Soil Edaphic Factors

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ABSTRACT

This study aimed to (1) Compare the nutritional characteristics and AOA (antioxidant activity) of clonal tea grown in lowland and highland plantations and (2) Investigate the influence of soil edaphic factors on tea foliar nutrition and AOA. Tea leaves and soils of fourteen tea clones were sampled between October 2021 to March 2022 from lowland and highland plantations in Peninsular Malaysia. Leaves were analysed for nutritional content and antioxidant activity, while soil samples underwent physical and nutritional analysis. Results showed significant variations in most foliar nutrients, except for Ca in the lowlands and Fe in the highlands. While the highland-grown tea exhibited higher nutrient concentration, lowland-grown tea demonstrated superior AOA. AT53 and 1248 clones were identified as promising among the clones, characterized by the highest nutrients and AOA levels, respectively. Soil nutrient availability significantly influenced foliar nutrient uptake, while soil pH was associated with AOA. Thus, the critical role of soil edaphic factors in shaping tea quality, providing valuable insight for tea growers to optimize soil and maintain tea yield and quality in the future. We found that soil nutrients have a significant association with nutrient uptake, while soil pH is associated with the agronomic characteristics of tea.

Keywords: AT53 and 1248 clones, Soil nutrients, Tea quality.

INTRODUCTION

Tea (*Camellia sinensis* L. (O) Kuntze) is a popular caffeinated non-alcoholic beverage around the globe and is currently consumed by 3 billion people (Pan *et al.*, 2022). It is

attributed to several health benefits in addition to its unique taste and aroma. The health-promoting properties of tea are due to the presence of polyphenols, particularly the flavan-3-ols, widely known as catechins, which are the main polyphenols in tea and have been reported to have a few derivatives

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(Lee *et al.* 2014). Epigallocatechin Gallate (EGCG) is the catechin derivative with the highest prevalence and pharmacological activity. EGCG is responsible for up to 70% of total catechins and has been proven to have chemo-preventive/chemotherapeutic actions against several malignancies (Steinmann *et al.*, 2013) and other disorders such as obesity, diabetes, neurological and cardiovascular diseases (Khan and Mukhtar, 2019). In addition to polyphenols, tea also contains minerals and trace element that play an important role in human metabolism. Regular consumption of tea may contribute to the daily dietary requirement of certain elements, such as manganese. Furthermore, having more potassium than sodium may benefit hypertensive patients (Fernandez *et al.*, 2002). Hence, both nutrient elements and polyphenols (particularly catechin, which has a strong antioxidant effect) are the main compounds that have great contributions to human health.

Variation in tea nutritional characteristics and AOA is associated with either internal (genetic make-up and stage of leaf development) or external factors, including season, altitude as well as soil physicochemical properties. For instance, earlier research revealed that certain nutrients, such as N, P, K and Mg (Xiang *et al.*, 2021) increase proportionally with altitude. Other researchers addressed this phenomenon as a result of growth reduction experienced by the plants grown in higher altitudes, which is highly associated with the highland's lower average temperature. This result is quite the opposite of tea AOA. Previous studies conducted by Owuor *et al.* (2011) and Martono *et al.* (2016) found that tea grown at lower altitudes tends to have better antioxidant performance. This indicates that higher temperature is one of the factors affecting catechin accumulation in plants grown at lower altitudes. Other environmental factors influencing the accumulation of tea catechin are blue light, water stress, shading treatment as well as soil physicochemicals, which act by modulating the expression of biosynthetic genes (Samynathan *et al.*, 2021). An earlier study by Zhao *et al.* (2017) discovered that the

contents of Na, Mg, Ca, Cr, Fe, Ni, Sr and Cd in tea leaves were significantly and positively correlated with those in topsoil or subsoil. It suggested a considerable rise of these elements in tea leaves when soil contents increased. Another study by Tseng and Lai (2022) in Taiwan revealed that soil characteristics, such as soil pH, exchangeable calcium and magnesium, significantly impact tea's free amino acid content.

Previous studies on the influence of soil edaphic factors on nutritional characteristics as well as AOA of tea have been conducted in Malaysia (Chan, *et al.*, 2007; Izzreen and Fadzelly, 2013; Amirah *et al.*, 2023), mostly in lowland plantations. In Malaysia, tea has been planted in two different altitudes for almost a century by BOH Plantation Sdn. Bhd., a Malaysian leading tea company. However, research on the influence of soil physicochemical factors, as well as altitude, on nutritional characteristics and the AOA of tea was less reported. Therefore, it is essential to determine the characteristics of distinct altitudes and each soil edaphic factor on the capabilities of tea clones in uptaking nutrients and accumulating catechin, as this may benefit growers in designing future strategies for sustaining tea yield and quality.

We hypothesized that soil physicochemical properties from different altitudes might play a major role in influencing the variation of foliar nutrient concentrations and the quality of tea. Therefore, in this study, we aimed to: (1) Compare the nutritional characteristics and AOA among tea clones from lowland and highland plantations; and (2) Investigate the association between soil physicochemical properties with nutritional characteristics and quality of clonal tea from both plantations.

MATERIALS AND METHODS

Study Sites

Highland tea clones were planted in Cameron Highland, Pahang, while lowland clones were grown in Bukit Cheeding, Selangor. The Cameron Highland is located

at approximately 1,400 m above sea level (asl), with an average temperature ranging from 18 – 25°C with an average humidity of 79–92% and 152–1,077 mm rainfall. The Bukit Cheeding Plantation is at a lower elevation, approximately 20 m asl, having a higher average temperature ranging from 28–31°C, lower humidity (74–86%), and rainfall (53.6– 596.3 mm) compared to the highland location.

Sample Collection and Analysis

Fourteen clones were sampled between October 2021–March 2022, from lowland and highland tea plantations. Leaf sampling sites were randomly selected (Table 1) and triplicated for young tea leaves (bud and the first three fully expanded leaves). Fresh tea leaves were cleaned with tap water and distilled water to remove the adhering materials before oven-drying at 60 °C for 4 days. The samples were then ground to get the fine powdered texture for further antioxidant and foliar nutrient analysis. Total N was analyzed using a CNS analyzer (CNSTruMax Determinator version 1.1x). For other foliar nutrients (total P, K, Ca, Mg, Al, and Fe), tea leaves were digested using the dry-ashing method following Alarefee *et al.* (2021) and, subsequently, analyzed by ICP–OES (Optima 8300, PerkinElmer,

USA).

Tea leaves were extracted using 80% aqueous methanol by digital ultrasonic bath following Afroz Bakht *et al.* (2019) with minor modifications using 40°C for 30 minutes before AOA analysis. The extract produced was evaluated for its Total Phenolic Content (TPC), DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and FRAP (Ferric Reducing Antioxidant Power) assay according to Amirah *et al.* (2023). The TPC was evaluated employing the Folin-Ciocalteu solution with gallic acid, which served as a reference. Ten-fold diluted tea extract (15 µL) was combined with distilled water (240 µL) and 0.25N Folin-Ciocalteu reagent (15 µL), and then mixed thoroughly. After 3 minutes of dark incubation at room temperature, 30 µL of 1N Na₂CO₃ was added. Following 2 hours of dark incubation at room temperature, absorbance was measured at 765 nm. In the DPPH assay, 20 µL tea extract was added to 180 µL of DPPH solution (150 µmol L⁻¹) and incubated for 40 minutes in darkness. Absorbance was measured at 517 nm, with 80% methanol used as blank. L-ascorbic acid served as the positive control. The antioxidant activity was expressed as an IC₅₀ value, calculated using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). In the FRAP assay, the FRAP

Table 1. Geographical locations of four clones sampled from both plantations.

Clones	Lowland plantation		Highland plantation	
	North latitude (°)	East longitude (°)	North latitude (°)	East longitude (°)
AT53	2.92450	101.57789	4.52340	101.39976
TV9	2.91870	101.57658	4.52110	101.40415
1248	2.92172	101.58327	4.52091	101.40018
2024	2.92813	101.58134	4.52322	101.39959
663	2.92231	101.58356	–	–
1294	2.92201	101.58292	–	–
2026	2.92368	101.58454	–	–
TBR2020	–	–	4.51790	101.41153
196	–	–	4.52321	101.39954
664	–	–	4.52281	101.39865



reagent needs to be freshly prepared. The reagent was made by mixing acetate buffer (0.3M, pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl₃ in a 10:1:1 ratio, then, warmed at 37°C. A mixture of FRAP reagent (280 µL) and tea extract (20 µL) was incubated at 37°C for 30 minutes and measured the absorbance at 593 nm.

The soils were sampled at 0–20 cm depth using an auger within 1–2 meters from each clone for soil physicochemical analyses. The soil samples were air-dried for several days and filtered through a 2 mm sieve before chemical and physical analysis (Alarefee *et al.*, 2021; Khairil and Burslem, 2018). Soil pH was measured at a soil: water ratio of 1:2.5. Soil total N was analyzed using CNS analyzer (CNSTruMax Determinator version 1.1x), while other soil nutrients (total P, K, Ca, Mg, Fe and Al) were analyzed using an ICP–OES (Optima 8300, PerkinElmer, USA) after the aqua regia extraction method (Alarefee *et al.*, 2021).

Statistical Analysis

All analytical results were performed as the average of three replicates using R-studio version 4.1 (Team R Core, 2021). Data were subjected to one-way ANOVA continued by Tukey's HSD to examine the variation of nutritional characteristics, AOA, and soil physicochemical properties. The significance level was set at a 95% confidence level ($\alpha=0.05$). In combination with Pearson's correlation, Principal Component Analysis (PCA) was performed to determine the influence of soil edaphic factors (nutrient concentrations and pH) on nutritional characteristics and the AOA of tea from both plantations. These were conducted using R-studio version 4.1 (Team R Core, 2021). PCA helps to identify patterns and relationships between variables by transforming the data into Principal Components (PCs) that summarize the dataset. Principal components were selected based on eigenvalues >1 and cumulative variance explained.

RESULTS

Nutritional Characteristics and AOA of Tea

The concentration of foliar nutrients differed significantly ($P < 0.05$) between lowland tea clones, except for Ca (Table 2). Among seven clones evaluated, AT53 had the highest foliar K ($1.84 \pm 0.38 \text{ mg g}^{-1}$), Mg ($0.80 \pm 0.16 \text{ mg g}^{-1}$), Fe ($13.00 \pm 0.79 \text{ mg g}^{-1}$) and Al concentration ($16.60 \pm 0.78 \text{ mg g}^{-1}$). Meanwhile, clones 2026 and 663 had the highest foliar N ($4.39 \pm 0.2\%$) and P concentration ($13.76 \pm 1.06 \text{ mg g}^{-1}$), respectively. In highland tea plantations, the majority of the foliar nutrition concentrations varied significantly ($P < 0.05$) among clones, except for Fe (Table 3). Of the seven clones evaluated, TBR2020 had the highest foliar Ca (3.89 mg g^{-1}), Mg (1.78 mg g^{-1}) and Al content (0.69 mg g^{-1}). Meanwhile, clones AT53, 1248 and 196 had the highest foliar N (5.18 %), P (8.35 mg g^{-1}) and K content (13.4 mg g^{-1}), respectively.

In terms of the AOA of tea, DPPH assay from lowland (Table 2) and highland (Table 3) plantations varied significantly ($P < 0.05$) among the clones. Clone 1248 ($50.66 \mu\text{g mL}^{-1}$) and TBR2020 ($127 \mu\text{g mL}^{-1}$) had the highest value for lowland and highland plantations, respectively. In addition, FRAP (Ferric Reducing Antioxidant Power) value significantly varied among the highland population only, with clone 1248 ($1.19 \text{ mM Fe (II) g}^{-1}$) having the highest value. However, Total Phenolic Content (TPC) displayed insignificant variations among tea clones from both plantations.

A PCA based on population mean values of seven nutrient elements and three antioxidative assays for tea grown in both plantations displayed the first axis explaining 76.97% of the variation in the data (Figure 1). This axis reflected a positive correlation with most of the variables (loadings 0.83 – 1.02), except N. The second PC axis that explained 8.45% variations, captured variation in N concentration

Table 2. Foliar nutrient concentration and antioxidant activity of lowland tea plantation.

Clone	Nutrient concentration							Antioxidant activity		
	N %	P	K	Ca	Mg	Fe	Al	TPC mg GAE g ⁻¹	DPPH µg mL ⁻¹	FRAP mM Fe(II) g ⁻¹
1248	4.22 ± 0.7ab	11.11 ± 0.4bc	0.26 ± 0.06b	0.61 ± 0.11	0.22 ± 0.07b	12.77 ± 0.8a	4.53 ± 1.2b	19.64 ± 0.15	50.66 ± 1.86c	2.10 ± 0.2
2024	3.79 ± 0.2ab	9.45 ± 0.5c	0.31 ± 0.12b	0.38 ± 0.04	0.14 ± 0.06b	6.01 ± 1.2b	16.19 ± 0.2a	19.29 ± 0.30	74.25 ± 2.79a	1.99 ± 0.1
AT53	3.55 ± 0.4ab	10.22 ± 1.0bc	1.84 ± 0.38a	0.70 ± 0.08	0.80 ± 0.16a	12.97 ± 1.4a	16.61 ± 1.4a	19.29 ± 0.26	74.36 ± 4.99a	1.55 ± 0.3
TV9	3.99 ± 0.6ab	11.85 ± 0.9ab	0.15 ± 0.04b	0.68 ± 0.09	0.11 ± 0.03b	10.26 ± 0.6a	3.66 ± 0.7b	19.30 ± 0.04	73.89 ± 4.31a	1.86 ± 0.3
663	2.97 ± 0.2b	13.76 ± 1.06a	0.55 ± 0.09b	0.58 ± 0.13	0.27 ± 0.06b	3.32 ± 0.8bc	4.43 ± 0.9b	19.03 ± 0.21	62.90 ± 4.93abc	1.90 ± 0.5
2026	4.39 ± 0.2a	9.19 ± 1.2c	0.47 ± 0.11b	0.64 ± 0.12	0.22 ± 0.003b	3.43 ± 1.3bc	3.72 ± 0.8b	19.23 ± 0.20	72.49 ± 1.97ab	1.65 ± 0.3
1294	4.57 ± 0.7ab	11.06 ± 0.5bc	0.24 ± 0.03b	0.38 ± 0.03	0.23 ± 0.04b	2.67 ± 0.4c	3.91 ± 1.7b	19.04 ± 0.10	54.61 ± 3.64b	1.76 ± 0.5
Mean	3.93 ± 0.2	10.95 ± 1.6	0.54 ± 0.13	0.57 ± 0.04	0.29 ± 0.05	7.35 ± 4.4	7.58 ± 5.8	19.26 ± 0.08	66.17 ± 2.40	1.83 ± 0.4
p-value	0.018*	0.000***	0.000***	0.116	0.000***	0.000***	0.000***	0.428	0.000***	0.551

The significance of the values is indicated as follows: * P < 0.05; ** P < 0.01; *** P < 0.001.

Table 3. Foliar nutrient concentration and antioxidant activity of highland tea plantation.

Clone	Nutrient concentration							Antioxidant activity		
	N %	P	K	Ca	Mg	Fe	Al	TPC mg GAE g ⁻¹	DPPH µg mL ⁻¹	FRAP mM Fe(II) g ⁻¹
1248	4.46 ± 0.1ab	8.35 ± 0.8a	11.1 ± 1.0ab	2.20 ± 0.1b	1.28 ± 0.1b	0.057 ± 0.003	0.38 ± 0.1ab	18.80 ± 0.94	140 ± 1.15a	1.190 ± 0.04a
2024	4.47 ± 0.3ab	7.98 ± 0.2ab	12.2 ± 0.5ab	2.42 ± 0.1b	1.59 ± 0.1ab	0.057 ± 0.01	0.30 ± 0.1b	17.34 ± 0.16	141 ± 2.38a	0.975 ± 0.05ab
AT53	5.18 ± 0.1a	6.74 ± 1.0ab	10.3 ± 1.4ab	2.63 ± 0.2b	1.74 ± 0.2ab	0.057 ± 0.01	0.36 ± 0.1ab	18.80 ± 0.49	133 ± 3.53ab	0.851 ± 0.05b
TV9	4.31 ± 0.6ab	5.78 ± 0.5b	10.8 ± 0.5ab	2.52 ± 0.3b	1.46 ± 0.02	0.050 ± 0.004	0.44 ± 0.1ab	18.35 ± 0.56	132 ± 2.34ab	0.989 ± 0.11ab
TBR2020	3.84 ± 0.1b	6.22 ± 0.3ab	10.9 ± 1.0ab	3.89 ± 0.3a	1.78 ± 0.1a	0.053 ± 0.01	0.69 ± 0.1a	16.97 ± 0.16	127 ± 2.99b	0.932 ± 0.05ab
196	4.59 ± 0.1ab	6.71 ± 0.3ab	13.4 ± 0.3a	2.62 ± 0.2b	1.91 ± 0.1a	0.060 ± 0.01	0.51 ± 0.1ab	17.80 ± 0.50	131 ± 0.58ab	1.180 ± 0.10a
664	4.74 ± 0.1ab	6.73 ± 0.1ab	8.55 ± 0.6b	2.25 ± 0.1b	1.47 ± 0.1ab	0.047 ± 0.003	0.22 ± 0.02b	16.58 ± 0.03	129 ± 3.43ab	0.801 ± 0.01b
Mean	4.51 ± 0.1	6.93 ± 0.3	11.03 ± 0.4	2.65 ± 0.1	1.60 ± 0.1	0.054 ± 0.003	0.41 ± 0.04	16.81 ± 0.31	133 ± 1.5	1.12 ± 0.07
p-value	0.049*	0.0454*	0.0347*	0.000***	0.009**	0.741	0.022*	0.051	0.014*	0.006**

The significance of the values is indicated as follows: * P < 0.05; ** P < 0.01; *** P < 0.001.



(loadings 0.83) of tea. Following the PCA, tea clones were clustered into two distinct groups. Clones with numbers 1–7 were from the lowland plantation and had a significant association with all the anti-oxidative assays, as well as foliar P, Fe and Al concentrations. Another cluster consisted of clones with numbers 8 – 14 originating from the highland plantation, and found to be highly associated with foliar K, Ca and Mg.

Four clones out of the ten clones evaluated were planted in both locations, namely AT53, TV9, 1248 and 2024. Even though they had similar genetic make-up, different geographical areas (altitude) and their microclimate influenced their physiology and metabolism. Based on our foliar analysis and PCA result, we found that four clones grown in both locations had similar clustered patterns as shown in Figure 1. Tea clones planted in the highlands tend to have higher foliar nutrients, particularly K, Ca,

and Mg. On the other hand, lowland-grown clones were associated with better antioxidant performance, as well as foliar P, Fe, and Al.

Soil Edaphic Factor

Soil P, K, Mg, Fe, and Al varied significantly among the seven lowland clones evaluated (Table 4). Soil obtained around clone 663 had the highest P (0.37 mg g^{-1}), Mg (0.39 mg g^{-1}) and Fe (10.20 mg g^{-1}) concentration. The highest soil K (1.17 mg g^{-1}) and Al (23.8 mg g^{-1}) concentrations were found from soil-derived near clones TV9 and 2026, respectively. In highland plantations, most of the soil nutrient content varied significantly, except for Ca (Table 5). Soil derived near clone 196 had the highest soil N (0.49%) and P (7.22 mg g^{-1}) concentration. The highest soil K (2.83 mg g^{-1}), Mg (3.10 mg g^{-1}), Fe (29.9 mg g^{-1}) and

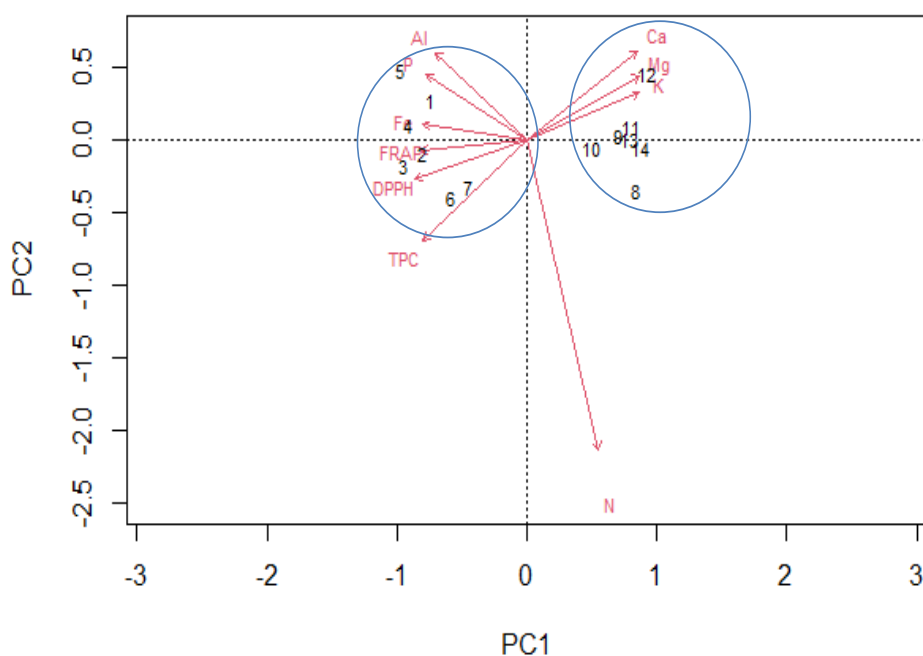


Figure 1. Biplots showing the distribution of 14 populations of tea along principal component axes 1 and 2 from PCAs summarizing variation in foliar nutrient elements and antioxidant activity assay (Note: 1–7= Lowland clones, 8–14= Highland clones).

Al (24.1 mg g⁻¹) concentration was obtained around clone TBR2020.

In terms of pH, both lowland (Table 4) and highland (Table 5) tea plantations had acidic pH, with averages of 4.23 and 3.58, respectively. The pH of lowland plantations differed significantly among clones with soil near clones AT53 and TV9 as the lowest and clones 1248 and 1294 as the highest.

Association of Soil Edaphic with Foliar Nutrient and AOA of Tea

We found that soil physicochemical properties had a significant association with foliar nutrients and AOA of tea (Table 6). Certain soil nutrients displayed direct correlation as well as inter-correlation with foliar nutrients: for instance, soil N with foliar N ($r= 0.55$), soil Ca with foliar Ca ($r= 0.77$), and soil Mg with foliar Mg ($r=0.59$). In addition, soil N is also significantly inter-correlated with foliar K ($r= 0.83$), Ca ($r= 0.64$), and Mg ($r= 0.76$). Soil P is significantly inter-correlated with foliar K ($r= 0.80$), Ca ($r= 0.61$), and Mg ($r= 0.69$). Both Ca and Mg demonstrated substantial inter-correlation with foliar K ($r= 0.90$; $r= 0.57$), while they showed an inter-correlation. Soil pH demonstrated a positive and significant correlation with foliar P ($r= 0.81$), Fe ($r= 0.53$), and Al (0.55). Furthermore, pH also had a positive and significant correlation with TPC ($r=0.59$) and AOA of tea, represented by DPPH ($r= 0.84$) and FRAP (0.78).

DISCUSSION

Foliar Nutrient and AOA of Lowland and Highland Tea Plantation

Our result demonstrated that clones grown in a highland plantation have a higher foliar nutrient content than those grown at a lower elevation. However, not all nutrients would increase proportionally with altitude. Our findings indicate that the foliar

concentrations of N, K, Ca, and Mg increase with altitude. This is similar to the result of a previous study. For instance, a recent study by Xiang *et al.* (2021) also found that foliar N and P content as well as leaf C: N ratio increase significantly with altitude. Several researchers assumed that the increase in leaf nutrient content of tea with altitude could be considered a consequence of biomass production decreasing with altitude, mainly due to the cold limitation in trees in mountainous regions (Jeyakumar *et al.*, 2020).

Highland plants tend to grow at a slower pace than those grown at lower altitudes. Decreasing temperatures, lower nutrient availability, and slower rates of photosynthesis are among the factors that influence the poor growth of highland plants (Jeyakumar *et al.* 2020). Generally, every 1 km increase of elevation results in a 6.5°C decrease in temperature. Low temperatures reduce soil microbial and enzymatic activity, thus limiting nutrient availability, hence, high-altitude soils are less fertile (Xu *et al.* 2015). Furthermore, lower air density and atmospheric pressure occurring at higher altitudes produced lower CO₂ levels and slower transpiration rates, which eventually led to lower rates of photosynthesis (Wang *et al.*, 2017). Therefore, previous researchers assumed that the increase in leaf nutrient content of plants with altitude could be considered as the plants' inability to use the absorbed resources for growth.

Nitrogen is the most abundant nutrient available in tea foliar from both plantations, followed by K. However, in some cases, tea may absorb more Ca than K (Hawkesford *et al.*, 2011). This is similar to our finding, especially in lowland plantations, where K ranked as the fourth macronutrient after Ca. Sufficient K greatly boosts the yield and quality of tea, as it speeds metabolism, triggers catechin synthesis, and promotes biotic and abiotic resistance by activating and governing several enzymes (Huang *et al.*, 2022; Ruan *et al.*, 2013). Meanwhile, Ca is beneficial for improving plant resistance, enhancing photosynthesis capacity, and

**Table 4.** Soil physicochemical properties of lowland tea plantation.^a

Clone	N	P	K	Ca	Mg	Fe	Al	Soil pH
	%				Mg g ⁻¹			
1248	0.11±0.009	0.20±0.030ab	1.04±0.076ab	0.37±0.018	0.35±0.029a	9.45±0.31abc	14.2±1.78b	4.35±0.25ab
2024	0.12±0.012	0.12±0.038b	0.83±0.118bc	0.23±0.023	0.20±0.006b	6.16±0.61c	20.2±1.68ab	4.27±0.03ab
AT53	0.08±0.028	0.19±0.034ab	0.51±0.026cd	0.33±0.003	0.18±0.009b	6.52±0.80bc	16.2±1.75ab	3.83±0.10b
TV9	0.10±0.028	0.30±0.047ab	1.17±0.030a	0.28±0.200	0.22±0.009b	8.69±0.96abc	20.8±1.93ab	3.86±0.15b
663	0.11±0.003	0.37±0.037a	1.08±0.094ab	1.04±0.401	0.39±0.027a	10.20±0.83a	22.1±1.78a	4.80±0.21a
2026	0.16±0.021	0.09±0.012b	0.39±0.021d	0.29±0.009	0.18±0.010b	9.78±0.98ab	23.8±1.41a	4.18±0.10ab
1294	0.11±0.030	0.37±0.090a	0.77±0.030bc	0.54±0.074	0.19±0.012b	7.27±0.51abc	18.1±1.22ab	4.35±0.25ab
Mean	0.11±0.007	0.24±0.029	0.83±0.065	0.44±0.08	0.24±0.019	8.30±0.41	19.33±0.87	4.23±0.09
P-value	0.166	0.003**	0.000***	0.058	0.000***	0.007**	0.012*	0.022*

^a The significance of the values is indicated as follows: * P< 0.05; ** P< 0.01; *** P< 0.001.

Table 5. Soil physicochemical properties of highland tea plantation.^a

Clone	N	P	K	Ca	Mg	Fe	Al	Soil pH
	%				Mg g ⁻¹			
1248	0.23±0.04ab	6.63±0.8a	0.83±0.03ab	1.44±0.13	1.03±0.07ab	15.5±2.1b	20.8±3.4ab	3.35±0.2
2024	0.42±0.05ab	2.16±1.8ab	0.29±0.19b	1.56±0.15	0.59±0.22ab	8.70±4.0b	8.64±4.2bc	3.77±0.2
AT53	0.27±0.02ab	1.50±0.1b	0.16±0.03b	1.58±0.02	0.41±0.05b	3.56±0.2b	7.68±0.4c	3.41±0.5
TV9	0.41±0.08ab	4.89±2.3a	0.51±0.12b	2.16±0.56	0.62±0.13ab	28.4±0.5a	21.3±1.1ab	3.57±0.2
663	0.14±0.06b	1.67±0.2b	2.83±1.15a	1.23±0.16	3.10±1.27a	29.9±4.5a	24.1±3.8a	3.73±0.01
2026	0.49±0.04a	7.22±1.8a	0.69±0.12ab	2.62±0.52	1.28±0.46ab	12.3±1.6b	11.4±1.3abc	3.79±0.3
1294	0.32±0.12ab	2.57±0.8ab	0.23±0.09b	1.50±0.40	0.40±0.15b	7.90±2.5b	4.81±1.4c	3.46±0.3
Mean	0.32±0.03	3.80±0.6	0.79±0.24	1.73±0.15	1.06±0.26	15.19±2.3	14.12±1.8	3.58±0.1
P-value	0.032*	0.039*	0.011*	0.128	0.029*	0.000***	0.000***	0.857

^a The significance of the values is indicated as follows: * P< 0.05; ** P< 0.01; *** P< 0.001.

Table 6. Pearson correlation coefficient (r) between soil nutrients and foliar nutrient concentration and AOA from both plantations.

Soil Variables	Pearson Correlation									
	Foliar N	Foliar P	Foliar K	Foliar Ca	Foliar Mg	Foliar Fe	Foliar Al	TPC	DPPH	FRAP
Total N	0.55*	-0.67**	0.83**	0.64*	0.76**	-0.73**	-0.62*	-0.67**	-0.79**	-0.60*
Total P	0.38	-0.56*	0.80**	0.61*	0.69**	-0.65*	-0.56*	-0.50	-0.74**	-0.38
Total K	-0.46	0.07	-0.02	0.27	-0.004	0.08	0.01	-0.12	0.09	0.13
Total Ca	0.41	-0.61*	0.90**	0.77**	0.86**	-0.80**	-0.69**	-0.71**	-0.82**	-0.64*
Total Mg	-0.05	-0.45	0.57*	0.77**	0.59*	-0.47	-0.40	-0.60*	-0.49	-0.39
Total Fe	-0.15	-0.42	0.45	0.61*	0.41	-0.40	-0.39	-0.42	-0.41	-0.30
Total Al	-0.060*	0.34	-0.40	-0.26	-0.47	0.26	0.25	0.44	0.38	0.49
pH	-0.62*	0.81**	-0.76**	-0.72**	-0.76**	0.53*	0.55*	0.59*	0.84**	0.78**
Soil PC Axes										
PC1	-0.51	0.78**	-0.96**	-0.86**	-0.91**	0.80**	0.71**	0.77**	0.92**	0.71**
PC2	0.47	0.01	-0.04	-0.30	-0.02	0.04	0.05	0.10	-0.02	-0.09

^a The significance of the values is indicated as follows: * P< 0.05; ** P< 0.01; *** P< 0.001.

promoting plant growth (Huang *et al.*, 2022). Magnesium is also involved in activating and governing several other physiological processes, such as photosynthesis, respiration and nucleic acid metabolism (Pongrac *et al.*, 2020).

Our study found that lowland plantations had a higher foliar Fe and Al content than

clones derived from highland plantations. This is suggested due to the higher foliar P in lowland over highland plantation, which is supported by the PCA result (Figure 1), with both the arrow of P and Fe going in the same direction. Iron (Fe) is an important micronutrient for plants since it is involved in metabolic activities such as DNA

synthesis, respiration, and photosynthesis. In contrast, Al is toxic to most plants since a micro-molar dose of it may inhibit root growth (Mahmud *et al.* 2024). However, tea plants are recognised as Al hyper-accumulators, which was initially coined for plants containing Al exceeding 1.0 mg g^{-1} dry mass (Chenery, 1955). However, distinguishing Al accumulators requires different thresholds depending on geographic origin: tropical plants need higher levels [$2.3 - 3.9 \text{ mg Al g}^{-1}$ of leaf dry mass (Metali *et al.*, 2012)]

In tea plants, Al concentration varies between young and mature leaves, whereas young leaves contain lower Al than mature leaves. Zhang *et al.* (2018) reported that Al concentrations in young leaves ranged from $0.25 - 0.66 \text{ mg g}^{-1}$, while in mature tea leaves, they ranged from $4.3 - 10.4 \text{ mg g}^{-1}$. A recent study revealed that Al concentrations in young leaves were between 0.67 and 2.21 mg g^{-1} , and in mature leaves between 2.63 and 7.83 mg g^{-1} (Zaman *et al.*, 2024). In our study, we found that young tea leaves were even able to accumulate up to 16.61 mg g^{-1} or 8-fold higher than the result of Zaman *et al.* (2024). Al primarily enters the plant root from acidic soils ($\text{pH} < 5.0$), where Al is solubilized into its toxic form (Al^{3+}). Organic acids like malate, citrate and oxalate are critical in Al detoxification. The formation of Al-organic acid complexes (such as Al-malate) is essential for their transport within the plant. These complexes are less toxic and can be transported through the symplastic pathway and loaded into the xylem for translocation to the shoots (Wang *et al.*, 2017). Thus, we assume that high Al accumulation of tea was due to the efficiency of detoxification and/or compartmentation mechanisms. Otherwise, such high Al concentrations would be extremely toxic. In the leaves, Hajiboland *et al.* (2013) revealed that up to 60% of Al in tea plants is stored in cell walls, primarily by binding to pectin and hemicellulose components. A significant portion of Al is sequestered in the vacuoles of tea leaves, reducing its toxicity to other cellular

organelles (Gao *et al.*, 2014). In addition, Al can be deposited in vacuoles that exist as complexes with phenolic substances, such as catechin (Barceló and Poschenrieder, 2002). P is among its components. Therefore, high Al uptake is generally coupled with high uptake of P, which was supported by our PCA result (Figure 1).

In terms of AOA, clones grown at a lower elevation tend to have a superior antioxidant, as demonstrated by greater TPC and FRAP values and lower DPPH IC₅₀ values. This result was similar to a previous study conducted in Africa (Owuor *et al.*, 2011), Taiwan (Chen *et al.* 2014), Indonesia (Martono *et al.*, 2016), and China (Wang *et al.*, 2022). Altitude increases are generally linked with decreases in tea polyphenol content (Ahmed *et al.*, 2019), whereas catechin is its major constituent. Catechin was reported to be inversely correlated to cultivation altitude as the EGCG (major catechin derivative) declined when the cultivation altitude was elevated (Chen *et al.*, 2014). Wang *et al.* (2022) confirmed the inhibition of catechin biosynthesis in high altitudes by stimulating the changes induced by temperature and light at different altitudes. Light intensity plays a major role in influencing the catechin content of tea. Xiang *et al.* (2021) proved that the catechin content and photosynthetic capacity of tea plants increased under optimum light intensities ($250 - 350 \mu\text{mol m}^{-2} \text{ s}^{-1}$). However, it will decrease under shading treatment ($150 \mu\text{mol m}^{-2} \text{ s}^{-1}$) or extreme high light intensity in highland plantation ($550 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Lowland-grown tea may produce up to 28% higher polyphenols compared to highland (Zhang *et al.*, 2018).

Association of Nutritional Characteristics and AOA with Soil Edaphic Factors

The PC1 of the soil properties (nutrient concentrations and pH) displayed a positive and significant correlation with foliar P, Fe, Al, and AOA (TPC, DPPH, FRAP). In PCA, PC1 is the first principal component that captures the maximum variance in the data. This suggested that PC1 represent soil fertility and nutrient availability gradient



that influences tea nutritional composition and antioxidant activity. A strong correlation between PC1 and foliar P or Fe suggested that PC1 reflects a soil axis related to P and Fe availability, essential for plant metabolism and enzymatic functions (Bhat *et al.* 2024). The presence of Al in the correlation might indicate soil acidity, which is common in tropical soil, further affects P availability and metal uptake (Ur Rahman *et al.*, 2024). Higher foliar TPC, DPPH, and FRAP linked to PC1 suggested that increasing soil P, Fe, and Al may enhance the synthesis of phenolic compounds, boosting antioxidant activity. This could be due to plant stress responses to metal presence (Fe and Al), increasing secondary metabolites such as phenolic. This suggests nutrient availability and potential soil stressors (such as Al) are key to plant biochemical composition.

Based on our analysis, total soil N was the most important element for tea growth and development, as it had a positive association not just with foliar N but also with foliar K, Ca, and Mg, as well as a negative correlation with foliar Fe and Al. In addition, total soil P, Ca, and Mg showed a substantial association with foliar K, Ca, and Mg, indicating that variations in tea's nutritional characteristics were influenced by the availability of nutrients. The results were similar to those of previous studies conducted in China (Zhao *et al.*, 2017) and Taiwan (Tseng and Lai, 2022). Nitrogen is an essential nutrient for tea production and accounts for 4-5% of tea leaf dry weight (Hamid *et al.*, 2014). Along with Mg and Mn, it up-regulated the expression of key genes for chlorophyll synthesis and promoted its synthesis (Chen *et al.*, 2021), thus resulting in a proportional increase in economic yield (Sitienei *et al.*, 2013).

Soil pH was the only soil parameter significantly correlated with foliar nutrients (P, Fe, and Al) and AOA of tea (TPC, DPPH, and FRAP assay). Soil pH from both plantations was highly acidic, with an average value of 4.23 and 3.58 for lowland and highland, respectively. Tea has also

been planted on acidic soil in Vietnam (pH 3.7-3.9) (Huu Chien *et al.*, 2018), Taiwan (pH 3.5 – 5.21) (Tseng and Lai, 2022) and China (pH 3.96-5.48) (Yan *et al.*, 2020). Soil pH significantly affects the availability of foliar P concentration. P tends to form insoluble complexes with Fe and Al in acidic soils, reducing availability. In contrast, as pH increases towards neutral, P becomes more available for plant uptake (Baquy *et al.*, 2024).

The increased Fe and Al uptake in tea plantations with increased soil pH was rare. Typically, Fe and Al solubility and availability decrease as soil pH increases (Ruan *et al.*, 2006; Alekseeva *et al.*, 2011). However, several mechanisms could explain this phenomenon. Tea plants have adapted to acidic soils and may release organic acids and chelating compounds from their roots, such as malate, citrate and oxalate. These compounds can solubilize Fe and Al even at higher pH by forming metal-organic complexes that remain plant-available. For instance, the formation of Al-malate is essential for transport within plants since these complexes are less toxic. Subsequently, they can be transported via the symplastic pathway and loaded into the xylem for translocation to the leaves (Wang *et al.*, 2017). Another mechanism includes microbial activity changes. An increase in soil pH could shift the microbial community structure. The shift had the potential to enhance siderophore-producing organisms that make Fe more available to plants (Choi *et al.*, 2024).

Higher soil pH has been associated with increased enzymatic activities in tea plants, which could enhance antioxidant defences. For instance, increasing soil pH from 3.3 to 5.3 enhances the nutrient availability in the rhizosphere. Subsequently, improving pH facilitates the uptake of essential elements including C, K, Ca, Mg, Mn, P and S, which play a vital role in phenolic biosynthesis (Jia *et al.*, 2024). The antioxidant activity, measured by DPPH and FRAP assay, tends to increase with higher soil pH. The improved nutrient uptake and enhanced

photosynthetic capacity under high pH conditions contribute to accumulating phenolic compounds, potent reducing agents. This results in higher FRAP values, indicating better antioxidant potential (Jahan *et al.*, 2022; Jia *et al.*, 2023).

CONCLUSIONS

We conclude that altitude and soil physicochemical properties are among the factors that influenced the variation of nutritional characteristics and the AOA of tea. Most foliar tea nutrients varied significantly among clonal teas, except Ca in the lowlands and Fe in the highlands. The highland tea population tended to have higher foliar nutrient concentrations, while the lowland population had better AOA performance. AT53 and 1248 were considered promising clones for having higher foliar nutrients and better AOA performance, respectively. Regarding soil edaphic factors, we found that soil nutrients and pH displayed a significant correlation with foliar nutrients, while soil pH was also significantly associated with the AOA of tea.

ACKNOWLEDGEMENTS

The authors are grateful to BOH Plantation Sdn. Bhd. for their invaluable cooperation and support in providing the sample subject for the research study. This study was funded by Geran Putra University Putra Malaysia (grant number GP/IPM/2020/9690400) and Tadam Ecoliving Research Grant (grant number 6300421-12038).

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غلظت عناصر غذایی برگ و فعالیت آنتی‌اکسیدانی چای (*Camellia*)
(*gsinensis* L. (O) Kuntze) کاشته شده در شبه جزیره مالزی و ارتباط آن با
عوامل خاکی

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چکیده

این پژوهش با هدف (1) مقایسه ویژگی‌های تغذیه‌ای و فعالیت آنتی‌اکسیدانی (AOA) چای کلونال کشت شده در مزارع پایین دست و مرتفع و (2) بررسی تأثیر عوامل خاکی بر تغذیه برگ چای و AOA انجام شد. برگ‌های چای و خاک چهارده کلون چای (tea clones) بین اکتبر ۲۰۲۱ تا مارس ۲۰۲۲ از مزارع پست و مرتفع در شبه جزیره مالزی نمونه برداری شدند. برگ‌ها از نظر محتوای غذایی و فعالیت آنتی‌اکسیدانی مورد تجزیه و تحلیل قرار گرفتند، در حالی که نمونه‌های خاک تحت تجزیه و تحلیل فیزیکی و تغذیه‌ای قرار گرفتند. نتایج، تغییرات قابل توجهی را در بیشتر مواد مغذی برگ، به جز کلسیم در مناطق پایین دست و آهن در مناطق مرتفع، نشان داد. در حالی که چای کشت شده در مناطق مرتفع غلظت مواد مغذی بیشتری را نشان داد، چای کشت شده در مناطق بیشتری AOA زیادتری را نشان داد. کلون‌های AT53 و 1248 به عنوان کلون‌های امیدوارکننده در بین کلون‌ها شناسایی شدند که به ترتیب با بالاترین مواد مغذی و سطوح AOA مشخص می‌شوند. در دسترس بودن مواد مغذی خاک به طور قابل توجهی بر جذب مواد مغذی برگ تأثیر گذاشت، در حالی که pH خاک با AOA مرتبط بود. بنابراین، نقش حیاتی عوامل خاکی در شکل‌دهی کیفیت چای، بینش ارزشمندی را برای چایکاران فراهم می‌کند تا خاک را بهینه کرده و عملکرد و کیفیت چای را در آینده حفظ کنند. ما دریافتیم که مواد مغذی خاک ارتباط معنی‌داری با جذب مواد مغذی دارند، در حالی که pH خاک با ویژگی‌های زراعی چای مرتبط است.